PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	22.11.2004
Applicant's or agent's file reference: B0131WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/08701	06.08.2003	08.08.2002

Applicant: CYTHERIS et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
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Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference B0131WO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/08701	International filing date *(day/month/year)* 06.08.2003	Priority date *(day/month/year)* 08.08.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/24		
Applicant CYTHERIS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.02.2004	22.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Sirim, P Telephone No. +49 89 2399-7732

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/08701

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-65 as originally filed

Sequence listings part of the description, Pages

1-18 as originally filed

Claims, Numbers

1-55 received on 17.05.2004 with letter of 07.05.2004

Drawings, Sheets

1/18-18/18 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8-30, 37, 44-53
	No:	Claims	1-7, 31-36, 38-43, 54,55
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-55
Industrial applicability (IA)	Yes:	Claims	1-55
	No:	Claims	

2. Citations and explanations

   see separate sheet

1. Documents

The following documents (D) have been taken into consideration; the numbering will be adhered to in the rest of the procedure:

D1: EP-A-0 314 415 (IMMUNEX CORP) 3 May 1989 (1989-05-03)

D2: WO 00 17362 A (SCHERING CORP) 30 March 2000 (2000-03-30)

D3: WO 96 04306 A (SCHERING CORP) 15 February 1996 (1996-02-15)

D4: WO 99 03887 A (BOLDER BIOTECHNOLOGY INC; COX GEORGE N III (US)) 28 January 1999 (1999-01-28)

D5: US-A-5 459 058 (LEDER PHILIP ET AL) 17 October 1995 (1995-10-17)

D6: WO 01 75140 A (UNIV CONNECTICUT) 11 October 2001 (2001-10-11)

D7: SRINIVASAN S ET AL: 'A model of IL-7 and extra-cellular domains of its receptor complex using distance geometry and structure-function data.' PROTEIN ENGINEERING, vol. 6, no. SUPPL., 1993, page 107 XP009003649, Winter Symposium on Advances in Gene Technology: Protein Engineering and Beyond; Miami, Florida, USA; 1993 ISSN: 0269-2139

D8: KROEMER ROMANO T ET AL: 'Prediction of the three-dimensional structure of human interleukin-7 by homology modeling.' PROTEIN ENGINEERING, vol. 9, no. 6, 1996, pages 493-498, XP002226699 ISSN: 0269-2139

D9: GOODWIN RG ET AL: 'Human interleukin 7: molecular cloning and growth factor activity on human and murine B-lineage cells' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, XP002077974 ISSN: 0027-8424

D10: COSENZA LARRY ET AL: 'Disulfide bond assignment in human interleukin-7 by matrix-assisted laser desorption/ionization mass spectroscopy and site-directed cysteine to serine mutational analysis.' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 52, 26 December 1997 (1997-12-26), pages 32995-33000, XP002226701

ISSN: 0021-9258

D11: COSENZA LARRY ET AL: 'Comparative model building of interleukin-7 using interleukin-4 as a template: A structural hypothesis that displays atypical surface chemistry in helix D important for receptor activation.' PROTEIN SCIENCE, vol. 9, no. 5, May 2000 (2000-05), pages 916-926, XP002226702 ISSN: 0961-8368

2. Subject-matter of the invention

The present application relates to the preparation and use of a specific IL-7 conformer which is considered as the native configuration of the protein. Said IL-7 conformer comprises three disulfide crosslinks formed by the (1) 1st and 4th cysteine, (2) 2nd and 5th cysteine and (3) 3rd and 6th cysteine of the protein.

3. Novelty (Art. 33(2) PCT) and Inventive step (Art. 33(3) PCT)

3.1. The subject-matter of the present claims 1 to 7, 31-36, 38-43 and 45-55 is not novel in the sense of Art. 33(2) PCT, since the isolated protein, its DNA and amino acid sequences, the existence of three intramolecular disulfide bonds and the therapeutic use of its immune stimulatory function have been disclosed in any of the documents D1 to D12.

The sole assignment of the positions of the cysteine bonds of IL-7 does not render a cloned protein novel, since the 3D structure of a protein is an intrinsic feature resulting from its primary structure.

Furthermore, based on computational methods and homology studies either of the documents D7 (figure) or D8 (page 497, §2) have proposed **the identical disulfide pattern** for IL-7 as the present application. Further, in D7 modeling studies showed that the receptor binding which is required for the biological function of IL-7 depends of this disulfide pattern and that improperly folded analogues of said molecule show altered receptor binding (D7: results/discussion). The authors of D8 consider this IL-7 form as the "unique" form (see abstract) which "unambiguously" results from the topology of the six cysteines within the molecule (page 497, §2).

Thus, the prior art is aware of the correct 3D structure of IL-7 including the exact positions of the 3 disulfide bonds required to obtain a properly folded protein capable of binding to its receptor.

The eukaryotic expression of the IL-7 as already disclosed in either of D1 (example 7), D2 (pages 51-56), D5 (columns 3-5) and D6 (example 7) will automatically result in a properly folded protein.

The method used in the present application for the bacterial expression of IL-7, comprising a first step of denaturation of the protein followed by a renaturation step resulting in a proper folded protein is just a standard method routinely followed by persons skilled in the art for the purification of proteins comprising intramolecular disulfide bonds.

Consequently, the subject-matter of the present claims 1 to 55 lacks an inventive step in the sense of Art. 33(3) PCT.

CLAIMS

1. An IL-7 drug substance comprising, as the desired product, an IL-7 conformer, wherein said conformer comprises the following three disulfide bridges: Cys: 1-4 (Cys2- Cys92); 2-5 (Cys34- Cys129) and 3-6 (Cys47-Cys141) and wherein said drug substance is substantially free of IL-7 molecular variants or product related impurities.

2. IL-7 drug substance according to claim 1, wherein said IL-7 conformer is a recombinant human IL-7 conformer.

3. IL-7 drug substance according to claim 2, wherein said IL-7 conformer comprises the amino acid sequence SEQ ID NO: 2 or 4.

4. IL-7 drug substance according to claim 1, wherein said IL-7 conformer is a recombinant simian IL-7 conformer.

5. IL-7 drug substance according to claim 4, wherein said IL-7 conformer comprises the amino acid sequence SEQ ID NO: 12.

6. IL-7 drug substance according to anyone of claims 1 to 5, wherein said IL-7 conformer is not glycosylated.

7. IL-7 drug substance according to anyone of claims 1 to 5, wherein said IL-7 conformer is glycosylated.

8. IL-7 drug substance according to anyone of claims 1 to 7, wherein said IL-7 conformer is associated to the hepatocyte growth factor as a heterodimer.

9. IL-7 drug substance according to anyone of claims 1 to 7, wherein said IL-7 conformer is functionally attached to a Fc portion of an IgG heavy

chain through a peptide hinge region, said IgG being a human IgG1 or IgG4.

10. IL-7 drug substance according to anyone of claims 1 to 7, wherein said IL-7 conformer is functionally associated to a Human Serum Albumin (HSA) or a portion of HSA as a fusion protein

11. IL-7 drug substance according to anyone of claims 1 to 10, said drug substance being substantially free of an other IL-7 conformer.

12. IL-7 drug substance according to anyone of claims 1 to 11, wherein the total amount by weight of IL-7 in said drug substance is at least 98% by weight, preferably at least 99.5% by weight.

13. A pharmaceutical composition comprising an effective amount of a drug substance according to any one of claims 1 to 12 and one or more pharmaceutically compatible carriers.

14. Pharmaceutical composition according to claim 13, wherein the pharmaceutically compatible carrier is selected from sucrose, trehalose and an amino acid.

15. Pharmaceutical composition according to claim 14, wherein the pharmaceutically compatible carrier is contained in an appropriate buffer to form an isotonic solution.

16. Pharmaceutical composition according to any one of claims 13 to 15, wherein said appropriate buffer has a pH range comprised between 5 to 7.5, preferably 6 to 7, even more preferably of 6.5.

17. A pharmaceutical composition according to claim 16, wherein said appropriate buffer is an organic salt selected from a sodium citrate buffer and an ammonium acetate buffer.

18. A pharmaceutical composition according to claim 13, wherein said composition is a lyophilized form.

19. A pharmaceutical composition according to claim 13, wherein said composition comprises a protein (preferably human serum albumin) and/or a surfactant (preferably Tween 80).

20. A pharmaceutical composition according to anyone of claims 13 to 19, further comprising an immuno-stimulating agent selected from a hematopoietic cell growth factor, a cytokine, an antigen and an adjuvant, or a combination thereof, for combined, separate or sequential use.

21. A pharmaceutical composition according to claim 20, wherein said hematopoietic cell growth factor is selected from the Stem Cell Factor (SCF), particularly the soluble form of the SCF, G-CSF, GM-CSF, Flt-3 ligand, IL-15 and IL-2.

22. A pharmaceutical composition according to claim 20, wherein the cytokine is selected from γ interferon, IL-2, IL-12, RANTES, B7-1, MIP-2 and MIP-1α.

23. A pharmaceutical composition according to anyone of claims 20 to 22, wherein said antigen is selected from a synthetic or natural peptide, a recombinant protein, a killed, inactivated or attenuated pathogen product, a lipid, a portion thereof and a combination thereof.

24. A pharmaceutical composition according to claim 23, wherein said antigen is selected from antigens derived from HIV, Varicella Zoster virus,

Influenza virus, Epstein Barr virus, type I or 2 Herpes Simplex virus, human cytomegalovirus, Dengue virus, Hepatite A, B, C or E virus, Syncytium respiratory virus, human papilloma virus, mycobacterium tuberculosis, Toxoplasma and Chlamydia.

25. A pharmaceutical composition according to anyone of claims 20 to 24, wherein said adjuvant is selected from any substance, mixture, solute or composition facilitating or increasing the immunogenicity of an antigen and able to induce a Th1-type immune response, such as CpG, QS21, ISCOM and monophosphoryl lipid A.

26. Pharmaceutical composition according to anyone of claims 13 to 25, for administration to a human patient for prophylactic or therapeutic stimulation of B or T lymphocyte development and proliferation, or for enhancement of global or specific immuno-reconstitution, or for enhancement of humoral or cellular immune response.

27. A pharmaceutical composition according to anyone of claims 13 to 25, to prevent or reduce opportunistic infections in immunodeficient patients.

28. A pharmaceutical composition according to anyone of claims 13 to 25, to prolong lymphopoiesis stimulation and/or to produce specific immune response and/or to broaden the repertoire of a specific immune response in human patients.

29. A pharmaceutical composition according to claim 26, 27 or 28, wherein human patients are immunodeficient patients, cancer patients, patients undergoing grafts, patients infected with a virus or a parasite, elderly patients or any patients having low CD4 count.

30. A pharmaceutical composition according to anyone of claims 13 to 29, wherein the effective amount of the drug substance is comprised between

about 3 to 300 µg/kg/day, preferably between 10 to 100 µg/kg/day, and in particular administered from once daily, to twice or three times a week down to once weekly.

31. A nucleic acid molecule encoding an IL-7 polypeptide, wherein said nucleic acid molecule comprises an altered Shine-Dalgarno-like sequence.

32. A nucleic acid molecule comprising a sequence selected from SEQ ID Nos: 1, 3, 12, 16, 18, 20 or 22.

33. A vector comprising a nucleic acid according to claim 31 or 32.

34. A recombinant host cell comprising a nucleic acid according to claim 31 or 32 or a vector according to claim 33.

35. A recombinant host cell according to claim 34, wherein said recombinant host cell is a human cell or a bacterial cell.

36. A recombinant host cell according to claim 35, which is *Escherichia coli* or *Bacillus Brevis*.

37. A recombinant host cell according to claim 35, which is a Chinese Hamster Ovary (CHO), HEK-293 cell line or a human stromal or epithelial cell line.

38. An antibody specifically immunoreactive with an IL-7 conformer as defined in anyone of claims 1 to 7.

39. A method of producing an IL-7 drug substance as defined in anyone of claims 1 to 12, the method comprising:

a) providing a sample comprising IL-7 polypeptides,

b) purifying an IL-7 conformer which comprises the following three disulfide bridges: Cys: 1-4 (Cys2- Cys92); 2-5 (Cys34- Cys129) and 3-6 (Cys47- Cys141) to produce an IL-7 drug substance, and

c) optionally, measuring or quantifying, in the drug substance, said particular IL-7 conformer.

40. The method of claim 39, wherein said sample is obtained from recombinant prokaryotic or eukaryotic host cells producing IL-7 polypeptides.

41. The method of claim 40, wherein said sample is (or derives from) a culture of prokaryotic host cells encoding an IL-7 polypeptide and further wherein the method further comprises, prior to step b):

i) treating said sample to cause a complete denaturation of said IL-7 polypeptides,

ii) optionally purifying the denatured polypeptide obtained in step i) and

iii) refolding the polypeptides.

42. The method of claim 41, wherein step i) comprises the dissolution of inclusion bodies in a denaturant buffer.

43. The method of claim 41 or 42, wherein step ii) is performed by hydrophobic chromatography, ion-exchange or inverse phase chromatography.

44. The method of claim 42, wherein said hydrophobic chromatography is implemented using HIC butyl.

45. The method of anyone of claims 41 to 44, wherein step ii) is carried out at a pH comprised between 6 and 9, preferably between 7 and 8,5 inclusive.

46. The method of anyone of claims 41 to 45, wherein said purification step b comprises the performance of an affinity chromatography.

47. The method of claim 46, wherein said affinity chromatography is performed on a column of sulfated polysaccharides.

48. The method of claim 47, wherein the sulfated polysaccharide is dextran sulfate or heparin.

49. The method of any one of claims 39 to 48, wherein the IL-7 conformer is characterized in the drug substance by Mass spectrometry, infra-red spectroscopy, NMR, by determining circular dichroïsm, by measuring the affinity toward a specific monoclonal antibody raised against said IL-7 conformer, or heparin affinity chromatography, and measured or quantified by ELISA, bioassay or the affinity of said IL-7 conformer for IL-7 receptor and any method of protein quantification if applied to the isolated conformer.

50. A method of controlling an IL-7-containing preparation, comprising determining the presence and/or relative quantity, in said preparation, of an IL-7 conformer as defined in any one of claims 1 to 9.

51. A method of producing an IL-7 drug substance or pharmaceutical composition, said method comprising (i) culturing a recombinant host cell encoding an IL-7 polypeptide, (ii) isolating said recombinant polypeptide to produce an IL-7 drug substance and (iii) optionally, conditioning said IL-7 drug substance to produce a pharmaceutical composition suitable for therapeutic or vaccine use, said method further comprising a step of identifying, characterizing or measuring, in said drug substance or pharmaceutical composition, the quantity and/or quality of an IL-7 conformer as defined in any one of claims 1 to 9 and, more preferably, a step of selecting the drug substance or pharmaceutical composition which

comprises, as the active ingredient, more than about 95%, preferably 98% of said IL-7 conformer.

52. A method according to claim 40 or 51, wherein IL-7 expression by the recombinant host cells is inducible, regulated or transient, so that the cell culture and IL-7 expression phases can be dissociated.

53. The method of claim 51 or 52, wherein the quantity and/or quality of said IL-7 conformer is determined by mass spectrometry-related methods, with or without tryptic digest, circular dichroism, NMR, specific monoclonal antibody analysis for disulfide bridges and/or conformation characterization.

54. Use of an IL-7 drug substance obtained by a method according to anyone of claims 39 to 49, for the manufacture of a pharmaceutical composition to induce a prolonged lymphopoiesis stimulation and/or to amplify an immune response.

55. Use of an IL-7 drug substance obtained by a method according to anyone of claims 39 to 49, for the manufacture of a pharmaceutical composition to prevent or treat a disease associated with an immunodeficiency.